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Term: antibody near5 chimeric near5 glycosylat$

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L6	antibody near5 chimeric near5 glycosylat$	16	L6
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L6: Entry 11 of 16 File: USPT Feb 3, 1998

DOCUMENT-IDENTIFIER: US 5714350 A
TITLE: Increasing antibody affinity by altering glycosylation in the immunoglobulin variable region

Detailed Description Paragraph Right (47):
To construct the glycosylated humanized and aglycosylated chimeric M195 antibodies, the genes for the respective variable domains were modified by site-directed mutagenesis (Maniatis et al., Molecular Cloning: A Laboratory Manual, 2nd Ed., (1989), Cold Spring Harbor, N.Y. and Berger and Kimmel, Methods in Enzymology, Volume 152, Guide to Molecular Cloning Techniques (1987), Academic Press, Inc., San Diego, Calif., which are incorporated herein by reference). The modified genes were inserted in the pVg1 expression vector and transfected into Sp2/0 cells together with the respective light chain containing vectors, as described (Co et al., op.cit.). Antibody-producing clones were selected, and antibody purified by protein A chromatography, as described (Co et al., op.cit.).

Detailed Description Paragraph Right (50):
While an -Asn-Xaa-(Ser/Thr)- sequence is necessary for N-linked glycosylation, not all such sequences are actually glycosylated. To determine if glycosylation at Ash 73 actually occurs and whether it affects the antibody binding affinity, this glycosylation site sequence was removed from the chimeric M195 antibody and a similar glycosylation site sequence was introduced into the humanized antibody. To remove the site from the V.sub.H region of the chimeric antibody, the Asn codon at position 73 was changed to a Gln codon. To introduce a potential glycosylation site into the V.sub.H region of the humanized antibody, the sequence in position 73-76 was changed from -Glu-Ser-Thr-Asn- (SEQ. ID NO:3) to the sequence -Asn-Ser-Ser-Ser- (SEQ. ID NO:4) that occurs in the chimeric V.sub.H region. Residues 73-75 represent the -Asn-X-(Ser/Thr)- glycosylation signal, while residue 76 was replaced because it has been reported that the amino acid immediately after the glycosylation site can affect the extent of glycosylation (Gavel and Heijne, Protein Engineering 3:433 (1990)). These amino acid alternations were achieved by site-directed mutagenesis of the respective genes. The altered V.sub.H region sequences were inserted into heavy chain expression plasmids, which were then transfected into Sp2/0 cells together with the respective light chain containing plasmids.

Detailed Description Paragraph Right (51):
Antibodies purified from the original murine M195 hybridoma and from the transfectants were analyzed by SDS-PAGE (FIG. 2). Under reducing conditions, the heavy and light chains of the various antibody constructs respectively migrate as bands of approximately 50 kDa and 25 kDa. The light chains of the chimeric and humanized antibodies migrate slightly differently because of the differing compositions of their V.sub.L domains. The heavy chains of the forms of the chimeric and humanized antibodies with potential V.sub.H glycosylation sites (FIG. 2, lanes 2 and 4) comigrate with the murine heavy chains (lane 1), while the heavy chains of the forms without potential V.sub.H glycosylation sites migrate slightly faster (lanes 3 and 5). Since the only amino acid differences between the two forms of the chimeric antibodies, and respectively between the two forms of the humanized antibodies, are the changes introduced at the glycosylation site, the most plausible interpretation of the mobility shifts is that the forms with the site migrate more slowly because of an attached carbohydrate group. Moreover, for the three heavy chains with the V.sub.H glycosylation site (lanes 1, 2 and 4), there is a small lower band comigrating with the heavy chains without the site (lanes 3 and 5), suggesting that a small portion of the heavy chain in these antibodies (about 10-20%) is not properly glycosylated at Asn 73. The appearance of heavy chain doublets in SDS-PAGE analysis of monoclonal antibodies has often been observed before, and is now shown to result from heterogeneity in glycosylation of the V.sub.H region.

Detailed Description Paragraph Right (52):
Direct binding of iodinated antibodies to determine affinity constants may be inaccurate, due to iodine atoms introduced into the binding region or denaturation during radiolabeling. Therefore, to accurately compare the binding affinities of the various antibody constructs, the unlabeled antibodies were allowed to compete with iodinated murine M195 for binding to HL60 cells, which express the CD33 antigen. Human serum, containing human IgG, was present in the reactions to inhibit non-specific and Fc receptor binding. The binding affinity of murine M195 has previously been measured as 2.times.10.sup.9 M.sup.-1 by Scatchard analysis (Co et al., J. Immunol. (op.cit.), and the same value was obtained from the competition of unlabeled murine M195 with iodinated M195 (FIG. 3A). The chimeric M195 antibody competes with the same efficiency as murine M195 (FIG. 3A), giving an affinity of 2.times.10.sup.9.This is consistent with expectation, since the chimeric antibody has the same V domain as the murine antibody. However, the humanized M195 antibody competed more effectively that the chimeric (or murine) antibody, displaying an about 8-fold increase in binding affinity (FIG. 3B). The chimeric antibody from which the V.sub.H glycosylation site had been removed competed as well as the humanized M195 antibody (FIG. 3C), that is, elimination of the glycosylation site increased the binding affinity 8-fold. Conversely, the humanized antibody into which we re-introduced a glycosylation site at Asn 73 competed with similar affinity as the original mouse antibody, showing that glycosylation decreased the binding affinity (FIG. 3D).

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Search Results - Record(s) 11 through 16 of 16 returned.

☑ 11. 5714350 . 13 Jan 95; 03 Feb 98. Increasing antibody affinity by altering glycosylation in the immunoglobulin variable region. Co; Man Sung, et al. 435/69.6; 424/133.1 435/477 435/70.21 435/71.1 530/387.3. A61K039/395 C12P021/08 C12P021/04 C12N015/13.

☐ 12. 5545405 . 03 Nov 94; 13 Aug 96. Method for treating a mammal suffering from cancer with a cho-glycosylated antibody. Page; Martin J.. 424/133.1; 424/130.1 424/143.1 424/172.1 424/174.1 435/320.1 435/70.3 435/71.1 530/387.1 530/388.1 530/388.22 530/388.73 530/388.75 530/389.1 530/389.6 530/389.7. A61K035/16 A61K039/00 A61K039/395 C07K016/00.

☐ 13. 5545404 . 03 Nov 94; 13 Aug 96. Method for treating a mammal suffering from a T-cell medicated disorder with a CHO-Glycosylated antibody. Page; Martin J.. 424/133.1; 424/130.1 424/143.1 424/173.1 424/174.1 435/320.1 435/70.3 435/71.1 530/387.1 530/388.22 530/388.73 530/388.75 530/388.8 530/389.1 530/389.6 530/389.7. A61K035/16 A61K039/00 A61K039/395 C04K016/00.

☐ 14. 5545403 . 23 Nov 93; 13 Aug 96. Method for treating a mammal by administering a CHO-glycosylated antibody. Page; Martin J.. 424/133.1; 424/130.1 424/135.1 424/136.1 424/138.1 424/143.1 424/147.1 424/150.1 424/159.1 424/172.1 424/174.1 435/320.1 435/70.3 435/71.1 530/387.1 530/388.1 530/388.22 530/388.73 530/388.75 530/389.1 530/389.6 530/389.7. A61K035/16 A61K039/00 A61K039/395 C07K016/00.

☐ 15. US 6350861 B1 . Mutant antibody that binds CD33, useful for diagnosis and treating, e.g., cancer, autoimmune diseases or viral infections comprises a mutation that eliminates a variable region framework glycosylation site in the parent immunoglobulin chain. CO, M S, et al. C07K016/30.

☐ 16. ES 2131507 T3, EP 481790 A, AU 9185914 A, CA 2053585 A, ZA 9108248 A, EP 481790 A3, AU 645355 B, JP 06090752 A, NZ 240249 A, US 5545403 A, US 5545404 A, US 5545405 A, EP 822255 A2, EP 481790 B1, DE 69130912 E . CHO cell line capable of producing antibodies - useful as immunosuppressa nts for treating T-cell mediated disorders cancer and viruses (e.g. HIV and herpes). PAGE, M J, et al. A61K035/16 A61K039/00 A61K039/39 A61K039/395 C07K015/00 C07K015/06 C07K015/12 C07K016/00 C07K016/28 C12N000/00 C12N005/06 C12N005/10 C12N015/13 C12P021/08 C12R001/91 C12N005/10 C12R001:91 C12P021/08 C12R001:91 C12N005/10 C12R001:91 C12P021/08 C12R001:91 C12N005/10 C12R001:91 C12P021/08 C12R001:91.

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